Abstract No. brom226

Design, Testing and Optimization of a Continuous Flow Cell for Time-Resolved FTIR Studies of Protein Folding in the Millisecond and Sub-Millisecond Time Domain

P. Bromberg, N. Marinkovic, M. Sullivan, J. Toomey, S-R. Ye, D. Rousseau and M.R. Chance, (Albert Einstein College of Medicine)

Beamline(s): U2B

ABSTRACT: We are investigating the evolution of structure during the early stages of protein folding, quantifying the timescales for the initial collapse and secondary structure formation. Using time-resolved FTIR microscopy and our rapid mixing continuous flow apparatus, changes in protein structure during refolding to the MG state can be evaluated by monitoring the amide I band (1600-1700 cm^{-1}) as a function of refolding time. Time-resolved spectra can be collected by focusing an IR beam (globar or synchrotron source) at various locations on a flow channel perpendicular to the direction of solution flow (timescale = solution velocity x distance from mixer). We designed and tested a continuous flow cell composed of two sandwiched ZnSe windows, one window with the time-resolved spectroscopy (TRS) groove (200 x 60 microns cut along its length, held fixed to our rapid mixer (50 microsecond dead time) in an aluminum block. A hole in a thin seal permits the solution to pass from the mixer to the TRS groove. For data collection at the low timescales of the U to MG state transition, we minimized the hole diameter and thickness of the seal to a third of its original value. Passage of solution through the seal now takes 167 microseconds (previously 2 ms). Using a flow rate of 1.2 ml/min per syringe and our modified seal, the accessible timescales for spectral collection of good signal-to-noise quality with a globar is from 0.3-6.8 ms. Improvements in data acquisition time and S/N quality will be investigated using synchrotron IR radiation.